VOL. 8, NO. 7, JULY 2013 ISSN 1990-6145
ARPN Journal of Agricultural and Biological Science


www.arpnjournals.com

ANTIBACTERIAL ACTIVITY OF *Sophora mollis* AGAINST *Escherichia coli* AND PHYTOCHEMICAL TESTS OF PLANT

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ABSTRACT

Sophora mollis has medicinal value. So this work was conducted to check its antibacterial activity against *Escherichia coli* and five different phytochemical tests (Reducing sugar, Flavonoids, Saponins, Alkaloids and Tannins) were done. Plant material was collected from Hazargangi Chiltan National Park, Quetta and was dried and grounded for further use. Leaves and stem were used for antibacterial and phytochemical tests for antibacterial activity the each plant part was soaked in 100 ml of aqueous and methanol extracts (Leaves and Stem) in three different concentrations (0.5, 1.0 and 1.5g/100ml) with 24 and 48hrs soaking duration. All concentrations and with all soaking duration inhibition zones were recorded. The maximum inhibition zone was in 1.5g concentration with 48hrs in methanol extract. In case of phytochemical tests all tests were positive. So because of its medicinal and now antibacterial effect this plant must consider for further antibacterial activities against other microorganisms. Underground parts should be studied. And other phytochemical tests are recommended.

Keywords: soaking duration, saponins, antibacterial, *E. coli*.

INTRODUCTION

Sophora mollis belongs to family Papillionaceae. It is a small deciduous shrub with dense hairy twigs. Leaves are 10-20 cm long. Flowers are yellow and pods are persistently silky. Flowering takes place in April to May [1].

Plants are used medicinally due to the presence of effective compounds for human body in many ways. Now-a-days medicinal plants getting more attention than past because of the presence of chemical compounds which are the main source of medicines as they produce definite physiological effects on human body [2, 3]. Plants can be used in different ways likewise for pharmaceutical purposes and nutritional uses. Primitive man makes efforts to distinguish the plants useful in the field of medicines or for nutritional purposes [4]. Phytochemical compounds play an important role to fulfill the life processes and energy needs of human beings [5, 6, and 7].

Sophora mollis is used medicinally. Decoction of roots is useful for headache; juice is used in sore eyes [1]. The seed are useful for destroying vermin. Wood is hard which is used as fuel. It is cultivated at rocks in dry valleys at 1200-2000m. It is also used as fodder, fuel wood, medicines and for roof thatching [8, 9]. It is used traditionally as fuel wood in Chitral and was a best soil binder [10].

Plants were used as remedies and treatment of diseases for centuries. Plants derived antimicrobials represent a vast untapped source of medicines. Plants were considered as the base of antibiotic production and modern medicines [11]. Plants have been used as a value able source of natural products for maintaining human health for a long period of time. The plant extracts and phytochemicals which had anti-microbial activity were significantly important in the field of medicines [12]. Bioactive compounds extracted from the medicinal plants have significant value due to their physiological activities [13]. *E. coli* was a normal part of micro biota of the lower gastrointestinal tract of mammals including human and usually exist as a harmless commensal [14].

MATERIAL AND METHODS

Plant material was collected from Hazargangi National Park, Quetta on 7th June 2011. Leaves and stem of *Sophora mollis* were dried separately at room temperature and then grounded. Material was used for different phytochemical tests and antibacterial effects. For the determination of phytochemicals 25g of grounded plant material was soaked with 100ml of water and methanol for aqueous and methanol extract for 24 hours then were filtered [15, 16 and 17].
The tests used were:

EXPERIMENT NO. 1

Antibacterial activity of *Sophora mollis*

The experiment was to determine the antibacterial effect of the extracts of *Sophora mollis* on *E. coli*. The following steps were applied during the laboratory work.

Sterilization

The key step for the successful determination of antibacterial activity of an organism is sterilization. So, sterilization was followed by two methods: Dry and wet sterilization.

Preparation of extracts

About 0.5, 1.0 and 1.5gm concentration of leaves and stem extract of *Sophora mollis* was soaked for 24 and 48 hours. Four samples of each concentration (0.5, 1.0 and 1.5 gm.) of grounded powder of leaves and stem were weight and were soaked separately into four 100 ml conical flasks and marked them as for 24 hours and 48

hours. Added 100 ml distilled water in two flasks and 100 ml of Methanol in other two flasks. After the completion of soaking duration of water and methanol extracts solutions were finally filtered and the Watmann's filter paper discs were dipped into the both aqueous and methanol filtrate with their respective concentration (0.5, 1.0 and 1.5g) and soaking duration (24 and 48hrs).

Preparation of solid medium and inoculation

Saline bacterial cultural (standardized inoculum): About 5 ml of saline solution (0.85 gm of NaCl in 100 ml) was taken in a screw cap test tube and the bacteria (*E. coli*) were incubated in it by means of a sterilized bacterial cotton swab. After shaking the test tube well the relative turbidity of the saline culture was matched with the corresponding McFarland barium Sulphate Solution.

Kirby-Bauer Antibiotic test Procedure was followed for antibacterial activity. Mueller-Hinton Agar (MHA) was used as media to grow and check the growth inhibition of test organism. After solidification the plates were heavily inoculated with standardized inoculums by means of cotton swab to ensure the confluent growth of the organism *Escherichia coli*. The discs were aseptically applied to the plates at standardized intervals and were left for Growth in incubator at 37^0C for 24 hours. The plates were further examined for the presence of growth inhibition zones, which indicated by a clear zone surrounding each disc. The susceptibility of an organism to a drug was determined by the size of this zone.

EXPERIMENT NO. 2

Phytochemical screening

A) Test for reducing sugar

0.5ml of plant extract was mixed with 5ml of boiling Fehling solution (A and B) in a test tube.

Fehling solution A

Take 6.93g $CuSO_4$ and dissolve in 100ml water.

Fehling solution B

Take 34.6g KNaCu + 10g NaOH and dissolve in 100 ml water.

B) Test for flavonoids

0.5 ml plant extract was added with 5ml of water, 5ml of dilute ammonia and 1ml of conc.H_2SO_4.

C) Test for saponins

0.5 ml plant extract was added with 5ml of water in a test tube and shake it vigoursly. A stable forthning was formed then add few drops of olive oil and shaked. An emulsion was formed.

D) Test for terpenoids

0.5 ml of plant extract was added with 2ml of chloroform and 3ml of conc. H_2SO_4.

E) Test for tannins

0.5 ml of plant extract was boiled with 10ml of water then filtered. Few drops of 0.1% ferric chloride were added.

RESULTS

i) Antibacterial activity tests

Leaves aqueous and methanol extracts of 0.5, 1.0 and 1.5g concentration with 24 hours soaking duration

Leaves aqueous and methanol extracts of Sophora mollis had antibacterial effect on E. coli growth. In case of aqueous extract the maximum inhibition zone (23.2 mm) was observed in 1.5g concentration with 24 hours soaking duration, while in case of methanol extract the maximum inhibition zone (15.6mm) was also recorded in 1.5g concentration with 24 hours soaking duration.

Leaves aqueous and methanol extracts of 0.5, 1.0 and 1.5g concentration with 48 hours soaking duration

Leaves aqueous and methanol extracts of Sophora mollis had antibacterial effect on 48hrs soaking duration. In case of aqueous extract the maximum inhibition zone (28.2mm) was observed in 0.5g concentration with 48 hours soaking duration.

While in case of methanol extract the maximum inhibition zone (16.4mm) was also recorded in 0.5g concentration with 48 hours soaking duration. Minimum inhibition zone was in 1.0g concentration in both aqueous and methanol extracts.

Stem aqueous and methanol extracts of 0.5, 1.0 and 1.5g concentration with 24 hours soaking duration

Stem aqueous and methanol extracts of Sophora mollis had antibacterial effects. In case of aqueous extract the maximum inhibition zone (13.2 and 13.6mm) was recorded in 0.5 and 1.0g concentration respectively with 24 hours soaking duration and 1.5g concentration was also recorded with inhibition zone of 11.6mm but less than the 0.5 and 1.0g concentration.

While in case of methanol extract maximum inhibition zone (12.4mm) was recorded in 1.5g concentration and 0.5 and 1.0g also had inhibition zone of 10.4mm but less than 1.5g concentration with 24 hours soaking duration.

Stem aqueous and methanol extracts of 0.5, 1.0 and 1.5g concentration with 48hours soaking duration

Stem aqueous and methanol extracts of 0.5, 1.0 and 1.5g concentration with 48 hours soaking duration also had antibacterial effect on E. coli. In case of aqueous extract the maximum inhibition zone (12.6mm) was in 1.5g concentration with 48 hours soaking duration and this antibacterial effect was followed with 0.5g concentration and then 1.0g concentration.

While in case of methanol extract maximum inhibition zone (19mm) was recorded in 1.5g

concentration and then was followed with 0.5 and 1.0g concentration respectively.

ii) Phytochemical screening of *Sophora mollis*

For the detection of photochemical in the leaves and stem of *Sophora mollis* 5 tests including test for reducing sugar, flavonoids, tannins, saponnins and terpenoids were performed. The results of these photochemical were as follows:

A) Test for reducing sugar

Dark green color appeared after the test which indicated the presence of reducing sugar in both parts of *Sophora mollis* (stem and leaves) in high concentration.

Figure-1. Test tube showing the result of reducing sugar.

B) Test for terpenoids

Terpenoids were also present in high concentration in the leaves and stem. Reddish brown color appeared which indicate the presence of terpenoids.

Figure-2. Test tube showing the result of terpenoids.

C) Test for tannins

Test for tannin showed different results in the leaves and stem of *Sophora mollis*. In the leaves it was present in very low concentration while in the stem it was absent.

Figure-3. Test tube showing the result of tannin.

D) Test for saponins

Saponins were observed in very low concentration in both the leaves and stem.

Figure-4. Test tube showing the result of saponins.

E) Test for flavonoids

Flavonoids were present in medium concentration in the leaves and stem of *Sophora mollis*.

Figure-5. Test tube showing the result of flavonoids.

DISCUSSIONS

This study was carried out to determine the antibacterial activity of the leaves and stem of *Sophora mollis* against *E. coli* and its phytochemical constitution. Different concentrations of the extracts of leaves and stem showed different inhibition zones against *E. coli*. The highest inhibition zone was observed in the leaves aqueous extract of 0.5g kept for 48 hours. The mean value of inhibition zone was of 28.2 ±2.638mm. The second highest inhibition zone was observed in the leaves aqueous extract of 1.5g kept for 48 hours the mean value was of 24 ±4.166mm.

In the methanol extracts of leaves the highest inhibition zone was observed in 0.5g extract of 48 hours (16.4 ± 3.826mm), while the second largest inhibition zone of methanol extract of leaves was observed in 0.5gm extracts of 48 hours (16.4 ± 3.826mm) [18].

In the stem the highest inhibition zone was observed in the methanol extracts of 48 hours (19 ±3.633mm). while in the aqueous extracts the high inhibition zone was reported in the 1g leaves extract of 24 hours (13.6 ± 1.356mm) [19]. The lowest inhibition zone was found in the 1g aqueous extracts of stem (9.2 ± 1.166mm). *Sophora mollis* was analyzed for phytochemical screening. Its photochemistry showed that different compounds were present with different concentrations. Leaves and stem were used for this purpose. Test of reducing sugar showed that it was present in high concentration a dark green color appeared which indicated the presence of reducing sugar the results agreed with the work of [20]. Test for terpenoids was practiced; reddish brown color appeared which the indication of its high concentration.

Test of tannins revealed different results in leaves and stem. In the leaves it was present in low concentration and showed light brownish red color the results follow the work of [15], while in the test of stem there was no color change which mean it was absent in the stem [21]. Flavonoids were present in a medium concentration; light yellow color appeared and indicated the presence of flavinoids. Saponins were present in very low concentration. An unstable forthning was formed and an emulsion was formed at the top of solution and indicated the presence of saponins [21].

Table-1. Mean and Standard error of leaf aqueous and methanol extracts of *Sophora mollis* with 24 hours (0.5, 1 and 1.5g) against *E. coli.*

Concentration in grams	Water	Methanol
	x□ ± ∂ (mm)	x□ ± ∂ (mm)
0.5	19.8 ± 5.153	13.2 ± 2.683
1	13.2 ± 3.692	13.2 ± 2.481
1.5	23.2 ± 5.215	15.6 ± 4.409

Table-2. Mean and Standard error of leaf aqueous and methanol extracts of *Sophora mollis with* 48 hours (0.5, 1 and 1.5g) against *E. coli.*

Concentration in grams	Water	Methanol
	x□ ± ∂ (mm)	x□ ± ∂ (mm)
0.5	28.2 ± 2.638	16.4 ± 3.826
1	16 ± 4.427	9.8 ± 1.326
1.5	24 ± 4.166	13.2 ± 2.712

Table-3. Mean and Standard error of stem aqueous and methanol extracts of *Sophora mollis with* 24 hours (0.5, 1 and 1.5g) against *E. coli.*

Concentration in grams	Water	Methanol
	x□ ± ∂ (mm)	x□ ± ∂ (mm)
0.5	13.2 ± 2.306	10.4 ± 1.85
1	13.6 ± 1.356	10.4 ± 1.019
1.5	11.6 ± 3.49	12.4 ± 1.624

Table-4. Mean and Standard error of stem aqueous and methanolic extracts of *sophora mollis* 48 hours (0.5g, 1g and 1.5g) against E. coli.

Concentration in grams	Water	Methanol
	x□ ± ∂ (mm)	x□ ± ∂ (mm)
0.5	10.2 ± 0.74	15.8 ± 2.135
1	9.2 ± 1.166	11.8 ± 2.77
1.5	12.6 ± 2.727	19 ± 3.633

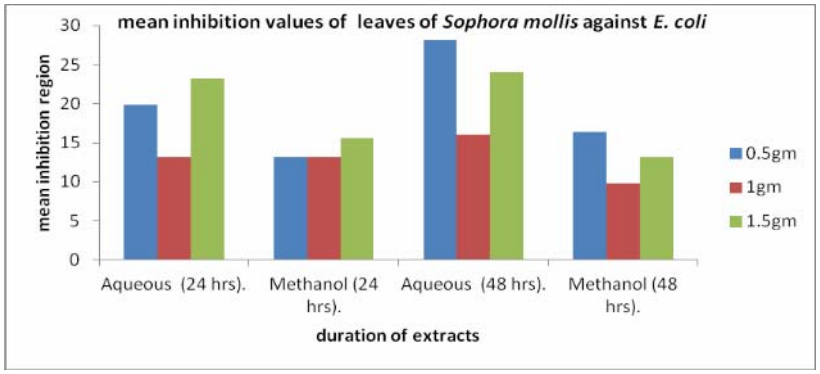

Figure-6. Mean inhibition region of the aqueous and methanol extracts of the leaves of sophora mollis against E. coli.

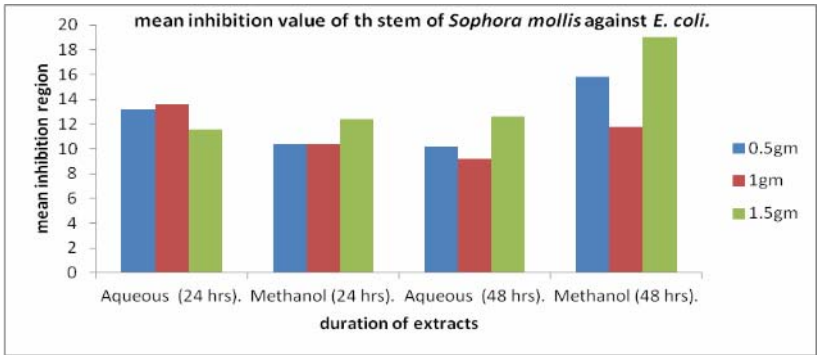

Figure-7. Mean inhibition region of the aqueous and methanol extracts of stem of *sophora mollis* against *E. coli.*

CONCLUSIONS

Finding confirmed the antimicrobial activity against *Escherichia coli* (local isolate). However, further research work like antimicrobial activity of different plant parts and essential oil is required to determine the effect of the extract on bacterial and other common skin infection including yeast.

REFERENCES

[1] Khan A. M and M. Aslam. Medicinal plants of Baluchistan. Qarshi industries pvt. Ltd. p. 33.

[2] Durrani M. J and M. Manzoor. 2006. Ethanobotanical study of some plants of SBK Woman's University, Quetta, Balochistan. Pakistan Journal of Botany. 12(1): 83-87.

[3] Durrani M. J., M, Manzoor and S. Irfan. 2009. Folk uses of some plants of Quetta, Pakistan. Pakistan journal of Plant Sciences. 15(1): 1-6.

[4] Shinwari Z. K. and S.S. Gilani. 2003. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). Journal of Ethno pharmacology. 84: 289-298.

[5] Hoffman P. C., Combs D. K. and Casler M. D. 1998. Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. Journal Dairy of Sciences. 81: 162-168.

[6] Mathew C. E and K.G. Ahern. 1999. Biochemistry. 3rd Edn. Benjamin Cummings.

[7] Dingma S. L. 2002. Water in soils. Infilteration and redistribution. Physical hydrogen, 2nd edition, upper saddle river, New Jersey, USA. Prentice-Hall, Inc. p. 646.

[8] Tareen R.B., T. Bibi., M.A. Khan., M. Ahmed and M. Zafar. 2010. Indigenous knowledge of folk medicines by the women of Kalat and Khuzdar regions of Baluchistan, Pakistan. Pakistan Journal 0f Botany. 42(3): 1465-1485.

[9] Durrani M.j., A. Razzaq., S.G. Muhammad. and F. Hussain. 2010. Floristic diversity, ecological characteristics and Ethnobotanical profile of plants of Aghberg rangelands, Baluchistan. Pakistan. Pakistan Journal of Plant Sciences. 16(1): 29-36.

[10] Awan M. R., M. Shah., G. Akbar and S. Ahmed. 2001. Traditional uses of economically important plants of Chitral, Malakand Division, NWFP, Pakistan. Journal of Botany (special issue). pp. 587-598.

[11] Massih A. R.., E. Abdou., E. Baydoun. and Z. Daoud. 2010. Antibacterial activity of the extracts obtained from *Rosemarinus officinalis*, *Origanum majorana* and *Trigoella foenum-graecum* on highly drug resistant Gram negative Bacilli. Journal of boy. 2010, 464087.

[12] Nescimento F.G.G., J. Locatelli., C.P. Freitas and L.G. Silva. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian journal of microbiology. 31: 347-256.

[13] Ebana R.U.B., B.B. Madunagu., E, D, Ekpe. and I.N. Otung. 1991. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *borresia ocymoides*, *Kola nitida* and *Citrus aurantifolia*. Journal of applied biotechnology. 71: 398-401.

[14] Cappuccino J.G and N. Sherman. 2007. Microbiology: A laboratory Manual. 7th Edition. Pearson Education. pp. 280-282.

[15] Siddique S., A. Verma., A. Rather., F. Jabeen and M.K. Meghvansi. 2009. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Advanced in biological research. 3: 188-195.

[16] Khan A. S., I. Ilahi., N. Hunar and H. Bibi. 2009. Preliminary phytochemical screening of some plants of ethanobotanical importance from district Gilgit, Northern, areas, Pakistan. Pakistan journal of plant sciences. 15(1): 15-18.

[17] Bibi Y., S. Nisa., A. Waheed., M. Zia., S Sarwar., S. Ahmed and M.F. Chaudhary. 2010. Evaluation of *Viburnum foetens* for anticancer and antibacterial potential and phytochemical analysis. African journal of biotechnology. 9(34): 5611-5615.

[18] Parekh J and S.V. Chanda. 2006. In-vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. Turk J. Bio. (31): 53-58.

[19] Mohsenzadeh M. 2007. Evaluation of antibacterial activity of selected Iranian essential oils against *Staphylococus aureus* and *Escherichia coli* in Nutrient Broth medium. Pakistan journal of biological sciences. 10(20): 3693-3697.

[20] Ayoola G.A., H. Coker., S. Adesegun., A. Bello., K. Obawey., E. Ezennia and T. Atangbayila. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. Tropical journal of pharmaceutical research. 7: 1019-1024.

[21] Achakzai A. K., P. Achakzai., A. Masood., S.A. Kayani and R.B. Tareen. 2009. Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. Pakistan Journal of Botany. 41(5): 2129-2135.